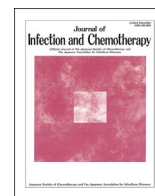




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Case Report

Potential for false-positive results with quantitative antigen tests for SARS-CoV-2: A case of a child with acute respiratory infection

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ABSTRACT

The quantitative antigen test based on the chemiluminescent enzyme immunoassay for SARS-CoV-2 has been used in international airports for quarantine in Japan. While cases of false-positive rapid antigen tests for SARS-CoV-2 were reported, false-positive cases of the quantitative antigen test with clinical information are rare. Here, we report a case of acute respiratory infection whose quantitative antigen test for SARS-CoV-2 was suspected to be false positive. A 9-month-old boy who presented with fever and rhinorrhea was admitted to our hospital under the Quarantine Act. He was diagnosed with COVID-19 based on the quantitative antigen test for SARS-CoV-2 performed at the quarantine station. None of the accompanying family members were positive for COVID-19. Nucleic acid amplification tests (NAAT) for SARS-CoV-2 were all negative, and multiplex polymerase chain reaction detected human rhinovirus or enterovirus infection. This case suggests that the results of the quantitative antigen test should be interpreted together with clinical information, and NAAT should be performed when false-positive results are suspected to avoid unnecessary isolation.

1. Introduction

A variety of rapid antigen tests (RATs) have been developed to confirm SARS-CoV-2 infection [1]. Of these, the quantitative antigen test based on the chemiluminescent enzyme immunoassay (CLEIA; Lumipulse G SARS-CoV-2 Ag Kit, Fujirebio, Tokyo) can quantitatively evaluate antigens in the nasal cavity or saliva with a relatively high accuracy in a short processing time. It was consequently approved for testing by the Pharmaceutical Affairs Law in June 2020 in Japan [2]. Because of its convenience, the rapid quantitative antigen test has been used for initial screening for quarantine in the international airports in Japan [3].

While they are convenient, false-positive results with RATs have been reported [4–6]. False-positive RATs results cause unnecessary isolation and increase in the burden of medical staff in facilities [4,5]. Several cases of false-positive RATs based on immunochromatography have been reported with detailed clinical information [4,5]; however, false-positive cases for the quantitative antigen test are rare. Only one false-positive case has been reported with patient information in the literature [6]. Here, we report a case of acute respiratory infection, with

possible false-positive result of quantitative antigen test for SARS-CoV-2, later suspected to have rhinovirus or enterovirus infection.

2. Case report

A 9-month-old boy who presented with fever and rhinorrhea was admitted to our hospital. He arrived in Japan from the Netherlands with his brother and mother. He had stayed with his family in his home and was not enrolled in childcare services. He had no contact with non-family members in the 2 weeks prior to arrival. He was diagnosed with COVID-19 based on the quantitative antigen test result from a nasopharyngeal swab performed in the quarantine station. The antigen value was 11.8 pg/mL. Both his brother and mother underwent the same quantitative antigen tests, and the results were negative. He stayed in the designated hotel under the Quarantine Act with his family. On the day when the test was performed, he was found to have rhinorrhea. Two days later, he developed a fever and was transported to our hospital. On admission, his general appearance was good, body temperature was 39.3 °C, and oxygen saturation on room air was 98%. Physical

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examination revealed no rash and no signs of pneumonia. Reverse transcriptase loop-mediated isothermal amplification (LAMP) for SARS-CoV-2 by nasopharyngeal swab was performed on hospital day 3 for him, his brother, and his mother, and all were negative. Real-time polymerase chain reaction (RT-PCR) for SARS-CoV-2 was also performed on days 4 and 5, and both were negative. We also performed multiplex PCR using a FilmArray Respiratory Panel 2.1 and detected human rhinovirus (HRV)/enterovirus, not SARS-CoV-2. The boy fully recovered and was discharged on hospital day 6.

3. Discussion

This case demonstrates the quantitative antigen test could return false-positive results, which subsequently requires additional confirmation by NAAT, especially when clinical information is not consistent with the test result.

The sensitivity and specificity of the quantitative antigen test for nasopharyngeal swabs were reported to be 91.7% and 99.6%, respectively, with the manufacturer's recommended cutoff value (positive >10 pg/mL; indeterminate 1.0–10 pg/mL, negative <1.0 pg/mL). In one study, a false-positive case with 27.3 pg/mL antigen value was observed, and this patient was diagnosed with COVID-19 by RT-PCR 4 days earlier [7]. Another false-positive case of the quantitative antigen test was reported for a 96-year-old woman with general malaise; however, the type of specimen or antigen value was not described [6]. In the present case, the antigen value was 11.8 pg/dL, which was just slightly above the cutoff value for a positive result. We suspected a false-positive result because none of his family members were found to have COVID-19 on repeat testing. In addition, a small child in this age group is less likely to get COVID-19 from non-family members unless he has had close contact with them. We could not perform NAAT on the same day when the quantitative antigen test was performed; therefore, the NAAT may have possibly turned negative by hospital day 4. However, this is less likely because LAMP and RT-PCR were performed not too long after the emergence of symptoms.

A two-step method combining CLEIA and NAAT is considered the best available testing method for mass screening [2]. In fact, this two-step methodology combining CLEIA and NAAT has already been implemented at international airports in Japan [3]. A previous study reported that CLEIA was performed as the first step for 88,924 international arrivals using self-collected saliva, and 254 (0.29%) were positive and 513 (0.58%) were indeterminate. Subsequently, NAAT was performed as the second step for 513 with indeterminate results, which confirmed 34 (6.6%) were positive and 479 (93.4%) were negative [3]. In the present case, NAAT was not performed because the value was not within the indeterminate range and was just slightly above the cutoff for positivity. This case suggests that NAAT should be performed to rule out false-positive results, especially when the antigen value is close to the cutoff, and clinical information does not suggest COVID-19.

False-positive RATs cases have been reported in pediatric patients with rhinovirus infections; however, cross-reactivity to rhinovirus has not been proven, and its relationship is unclear. One study reported three pediatric false-positive cases with the rapid antigen test Espline SARS-CoV-2 (Fujirebio, Tokyo, Japan) with rhinovirus infections. In these cases, film array revealed HRV/enterovirus infections, and human rhinovirus typeA was confirmed by RT-PCR with sequencing [5]. Another study reported three pediatric patients tested false positive for the same rapid antigen test and were later found to have had rhinovirus infections [4]. Additionally, in the present case, details regarding the type of the virus were not confirmed, but the patient was suspected to have either rhinovirus or enterovirus infection. However, the package insert for the rapid antigen test stated no proven cross-reactivity to human rhinovirus [8]. A high specimen viscosity and interference of

human antibodies are believed to be factors causing false-positive antigen tests through non-specific reactions [9,10]. The possibility that those viral infections may induce non-specific reactions other than direct cross-reactivity, thereby indirectly resulting in a false-positive result, should be evaluated in the future.

Our case report confirms the possibility of obtaining a false-positive quantitative antigen test for SARS-CoV-2. The test results should be interpreted along with clinical information, and NAAT should be performed when false-positive results are suspected to avoid unnecessary isolation.

Authorship statement

RH wrote the original draft. TW and YO conducted the laboratory analyses. NO and YN were involved in the patient care. All the authors reviewed the manuscript and approved the final version of the manuscript.

Consent

Written informed consent was obtained from the patient's parent for publication of this case report.

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Declaration of competing interest

The authors have no conflicts of interest to declare.

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